

Dentigerous bones and dentition in the paedomorphic plethodontid salamander *Eurycea neotenes*¹

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Tooth-bearing bones and dentition of males and females of the paedomorphic plethodontid salamander *Eurycea neotenes* are described using alizarin transparencies and SEM micrographs. Dentition is strictly monostichous at the upper jaw (premaxillae only), the lower jaw (dentaries, splenials) and the palate (vomeres, palatopterygoids). Teeth in both sexes are monocuspid, conical, and pedicellate, but the weak zone between the dentine crown and the pedicel is not very distinct and does not progress beyond a late larval stage. In addition, we found some sex-linked characters. Compared with females, males possess stronger premaxillae with separated *processus faciales*, fissured inner margins of the dentated parts of the palatopterygoids that overlap the parasphenoid, and larger splenials bearing more teeth. In addition, males appear to have longer teeth, especially on the anterior portion of premaxillae.

INTRODUCTION

In Urodela, tooth bearing bones, dentition and the form of teeth undergo a more or less specific developmental sequence and remodeling during metamorphosis (WILDER, 1925; STADTMÜLLER, 1924; GRIVIN, 1988; CLEMEN & GRIVIN, 1977, 1994). Paedomorphic species often possess a mosaic of larval and metamorphic traits which reflect their "degree" of paedomorphosis, such traits can be found also in the tooth systems (e.g., GRIVIN & CLEMEN, 1980; CLEMEN & GREVEN, 1988; REILLY 1994).

In plethodontids, paedomorphosis has played a significant role in influencing evolutionary patterns and larval as well as paedomorphic features of the tooth systems have been described (e.g., LARSEN, 1963; WAKE, 1966, MUTZ & CLEMEN, 1992).

1. Dedicated to Prof. Dr H. Hartwig, Cologne, on the occasion of his 90th birthday
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In the present paper we examine the lower and upper jaw as well as the palate of the plethodontid *Eurycea neotenes*. This species was considered to be probably paedogenetic (progenetic) and not neotenic (BRUCE, 1976; SWEET, 1977; for terminology and the use of the term paedomorphosis to specify any retention of juvenile features by adult descendants, see GOULD, 1977; WAKE, 1980; REILLY, 1994). Our observations broaden the hitherto available studies on this topic in the genus *Eurycea* (see STEWART, 1958; LARSEN, 1963; WAKE, 1966; SWEET, 1977; MUTZ & CLEMEN, 1992) and draw the attention to some sexual dimorphic characters.

MATERIALS AND METHODS

A total of 5 adults of *Eurycea neotenes* (3 females, 2 males) of unknown origin were examined. Specimens that died in captivity were preserved in 70 % ethanol. Total length of males was approximately 68 mm, snout-vent length approximately 33 mm with only a negligible range. The respective measurements in females were 60 mm and 34 mm. Males had three lobes per testis and in females vitellogenesis was in progress or finished. According to BRUCE (1976), males therefore were in the third reproductive year or fourth year of life and females were maturing or mature.

Specimens were postfixed in Lillie buffered formalin for 3 days. Cleared specimens were stained only for bone employing the method of PARK & KIM (1984).

After drawing, the skeletal structures of the head of the specimens were transferred into an enzyme solution of 30 ml saturated aqueous sodium borate, 70 ml distilled water and 1 g pancreatin for several days to remove the soft tissue. Then the delicate dentigerous bones were extracted, dehydrated in ethanol, critical-point dried and mounted on metal plates. They were sputter-coated with gold and viewed in a SEM (Hitachi S-530).

The length of the head of two males and two females was measured from the midst of the premaxillary arcade to the posterior end of the parasphenoid and from the premaxillae to the condylus. The width of the parasphenoid was determined at the level of the quadrate. Teeth were measured directly on the SEM micrographs.

RESULTS

Length of the male's head was 6 mm from the premaxillae to the posterior end of the parasphenoid and 6.5 mm to the condylus. The largest width of the parasphenoid was 2.2 mm. In the females the head measured 5 mm and 5.5 mm in length, and the parasphenoid 2.0 mm in width.

The upper jaw is composed only of the unpaired (fused) premaxilla. Maxillae are entirely lacking. The tooth bearing *partes dentales* of the premaxillae form only a short arcade. Two long *processus faciales* (*p. praemaxillares* according to CLEMEN & GREVEN, 1994) arise

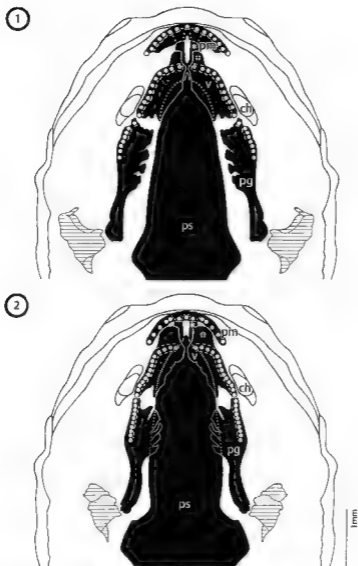


Fig 1 Ventral view of the anterior part of the skull of an adult female of *Eurycea nictitans* showing the dentigerous and non dentigerous dermal bones of the upper jaw and the palate (black) and the ossified quadrate (hatched). ch, choana, pg, palatopterygoid, pm, premaxillae with anteriorly separated *processus faciales* (*ppr. premaxillaris*) (asterisk), ps, parasphenoid, v, vomer with anterior process (white point). The posterior portion of the parasphenoid and other cartilaginous and ossified elements of the skull are omitted.

Fig 2 Ventral view of the anterior part of the skull of an adult male of *Eurycea nictitans*. For further explanations and abbreviations see fig. 1. Compared with the female (fig. 1), the *pars palatina* of premaxillae (pm) is missing, the *processus faciales* (asterisks) are separated along the entire length, the vomers possess larger anterior processes (white point) and the fissured inner margins of the dentated parts of the palatopterygoid (pg) overlap the broad parasphenoid (ps).

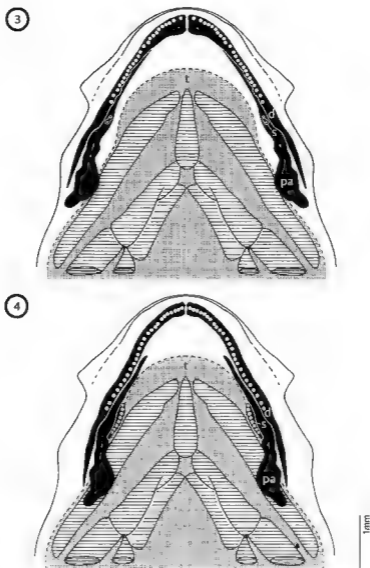


Fig 3 Dorsal view of the dentigerous and non dentigerous dermal bones of the lower jaw (black) and parts of the mostly cartilaginous hyobranchial apparatus (hatched) of an adult female of *Eurycea neotenes*. d, dentary; par, prearticular; s, splenial, t, tongue

Fig 4 Dorsal view of the lower jaw (black) and parts of the hyobranchial apparatus (hatched) of an adult male of *Eurycea neotenes*. For further explanations and abbreviations, see fig 2. Compared with the female (fig 3), the splenials (s) are larger and the anterior portions of the dentaries (d) are smaller

immediately below the tooth bearing portion (fig. 1-2). Two third of these processes are fused posteriorly in females; in males they are separated for their entire length (fig. 5-6) The toothless bony ledge along the lingual side (*pars palatina*) of the premaxillae is relatively large in females, but absent in males (fig. 1-2).

The ventral components of the skull include fully ossified paired vomers anteriorly and a median large parasphenoid posteriorly (fig. 1-2) The anteriorly directed processes of the vomers are small in females, but broad in males (fig. 10). In the former they do not attach to the premaxillae, in the latter they do and cover a larger portion of the *processus faciales*.

The posterolateral margin of each vomer forms approximately two thirds of the bony margin of the inner choana. The inner toothless *partes palatinae* of the vomer overlap the anterior part of the parasphenoid (fig. 7, 10) Vomers are separated widely from each other posteriorly (fig. 1-2). The ossified paired palatopterygoids begin at the posterior margin of the choanae and extend backwards to, but do not articulate with, the inner surface of the ossified quadrate. In one specimen, however, an adhesion to the quadrate was observed on the left side. The inner margin of the palatal portion of the palatopterygoid is highly fissured in both sexes (see fig. 7) This is obviously more pronounced in males, where this region overlaps the broad parasphenoid. In two females the fissured region was not completely ossified on one side, leaving separated bony patches (not pictured).

The lower jaw is composed of the dentaries that both form a broadly rounded arch. The lingual side of the Meckel's cartilage is invested by the prearticular (fig. 3-4). In females, dentaries are broader at their anterior end, the prearticulars extend more anteriorly than in males. A small splenial (coronoid) occurs on the inner margin of each dentary overlying the prearticular, it is very minute in females (fig. 8) and longer in males (fig. 9)

Premaxillae, dentaries, splenials, vomers and the palatal portions of the palatopterygoid bear strictly monostichous teeth (fig. 2-3, 7, 9-10) Teeth vary considerably in height. Tooth height depends on the site of attachment and on sex. Teeth of males are longer than those of females. These differences appear to be most obvious in the most anterior premaxillary teeth. In addition, males have more tooth loci at the vomers, the palatal portions of the palatopterygoids and, in particular, at the splenials (tab. 1) The small number of animals examined, however, does not allow further interpretation of the measurements and counts of functional teeth (tab. 1).

Teeth are separated from each other and are slightly recurved towards the esophagus (fig. 6, 11) They are similar in form, but decrease gradually in height and size posteriorly. Teeth are ankylosed at the jaws in a distinct pleural condition (fig. 11) and at the labial face of the vomers in a slight pleural condition (fig. 10). The majority of teeth of the labial face of the palatal portion of the palatopterygoid is attached horizontally (females) or more or less pleurally (males). Only a few (2-3) posterior teeth in males are attached horizontally (fig. 7, right side), whereas in females some anterior teeth are attached pleurally due to the different decrease of height of the *pars dentalis*.

Each tooth is composed of a monocuspid crown and a pedicel (fig. 5-12). Both elements are connected by collagenous fibres (fig. 13). A prominent dividing zone, however, as in most transformed urodelan teeth consisting of a broad lingual and a small labial deepening is

missing (fig. 11-12) Some teeth show resorption pits mainly in the pedicel and signs of wear apically. All teeth have at their lingual base one large opening to the pulp cavity and additional small holes around the base (fig. 7-8, 11).

DISCUSSION

In *Eurycea neotenes*, the developmental state of dentigerous bones and teeth does not progress beyond the larval condition. Similar traits in the tooth systems were found also in other paedomorphic taxa (for review, see GREVEN, 1988; see also CLEMEN & GREVEN, 1977, 1988; GREVEN & CLEMEN, 1979).

Maxillae, which seem to appear relatively late during ontogeny in plethodontids (WILDER, 1925, WAKE, 1966), and processes forming the posterior vomerine tooth patches that are typical for advanced metamorphosed plethodontids (STEWART, 1958, WAKE, 1966, MUTZ & CLEMEN, 1992; see fig. 12 in CLEMEN & GREVEN, 1994), are completely missing. Both structures are, however, present in transformed *Eurycea neotenes* (see below).

We found no signs of disintegration of the palatopterygoids. This disintegration is considered as one of the key factors indicating the onset of metamorphosis by some authors (REILLY, 1986, 1987, REILLY & ALTIG, 1996). Obviously the disintegration of the splenials runs parallel to that of the palatopterygoid, but dental laminae of the palatal portion of the palatopterygoid as well as of the splenials degenerate far earlier, indicating likewise the onset of metamorphosis (GREVEN & CLEMEN, 1985; MUTZ & CLEMEN, 1992).

The larval condition holds also for the dentition. In our specimens, dentary, premaxillary, vomerine, palatal and splenial teeth are strictly monostichous. Generally, in early urodelan larvae, at least the palatal portion of the palatopterygoid and the splenials bear more than one line of teeth, thus possessing tooth patches ("Zahnfelder"). In transforming *Eurycea* species, reduction of the dentition starts with the formation of a monostichous

- Fig 5 - Fused monostichously dentated premaxillae of a female of *Eurycea neotenes* with partially fused *partes faciales*
- Fig 6 - Fused monostichously dentated premaxillae of a male of *Eurycea neotenes*. The *partes faciales* are separated for their entire length.
- Fig 7 - Vomer (asterisk) and palatal portion of the palatopterygoid (point) of a male of *Eurycea neotenes*. Note the only slight pleural attachment of teeth and the fissured palatal portion (arrowhead).
- Fig 8 - The minute splenial of the female of *Eurycea neotenes* bears only one tooth and overlies the prearticular
- Fig 9 - The splenial of the male of *Eurycea neotenes* is larger having up to eight tooth loci
- Fig 10 - Vomer of the female of *Eurycea neotenes* with anterior processes (asterisk) and the labio-marginal tooth-line
- Fig 11 - Distinct lingual dividing zone of vomerine teeth, male of *Eurycea neotenes*. Note the openings to the pulp.
- Fig 12. - Dividing zone of a premaxillary tooth, labial side, male of *Eurycea neotenes*.
- Fig 13 - Collagenous fibers of the labial dividing zone of a dentary tooth, female of *Eurycea neotenes*

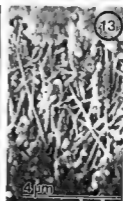
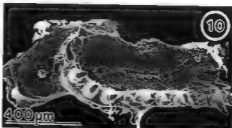
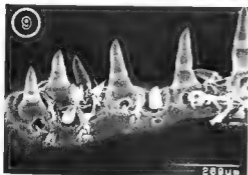
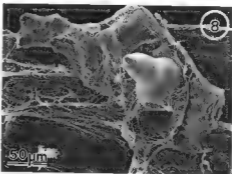
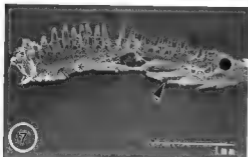
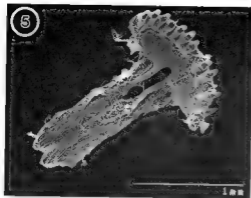


Table 1 -- Height of teeth and number of tooth loci per side in two males and two females of *Eurycea neotenes* as measured on SEM micrographs. Labial height is the distance from the margin of the bone to the tip of the tooth crown, lingual height the distance from the base of the pedicel to the tip of the crown.

Dentigerous bones		Height of teeth in μm		Number of tooth loci (per side)	
		Male	Female	Male	Female
Premaxillae					
Labial	Anterior	325-336	234-254	13-14	14-16
	Posterior	212-245	205-217		
Lingual	Anterior	407-415	325-336		
	Posterior	299-340	387-230		
Vomers					
Labial	Anterior	256-287	227-233	12-14	10-12
	Posterior	228-238	183-190		
Lingual	Anterior	349-354	247-262		
	Posterior	310-315	206-213		
Palatopterygoids					
Labial	Anterior		120-125	9-11	6-8
	Posterior		87-94		
Lingual	Anterior	222-230	175-182		
	Posterior	147-166	138-141		
Dentaries					
Labial	Anterior	248-252	195-203	24-26	23-25
	Posterior	234-241	196-205		
Lingual	Anterior	309-328	308-315		
	Posterior	245-257	219-224		
Splentals		165-205	126-130	7-8	1-2

pattern and ends with the complete disintegration of these bones (WILDER, 1925, MUTZ & CLEMEN, 1992). Therefore monostichy on the palatal portions of the palatopterygoids and the splentals is regarded as a late larval state. In contrast to other larval urodeles (see CLEMEN & GREVEN, 1977, 1994, GREVEN & CLEMEN, 1985, MUTZ & CLEMEN, 1992, AMIND & GREVEN, 1996), vomerine teeth even of early plethodontid larvae (and paedomorphic plethodontids) hitherto investigated are strictly monostichous (WAKI, 1966, MUTZ & CLEMEN, 1992). Thus, development of the vomerine dentition differs considerably from the general urodelan scheme. Contrary to transformed urodeles, teeth of the vomer and the palatine are ankylosed at the outer (labial) face of the bones, emphasizing again the late larval condition (for review, see CLEMEN & GREVEN, 1994).

Also form and size of the teeth are undoubtedly larval. Teeth are slightly recurved, monocuspid, conical and show a dividing zone that never reaches the condition found in transformed teeth. The typical developmental sequence documented in urodelan teeth is from undivided monocuspid to divided monocuspids in the late-stage larvae to divided bicuspids teeth during or immediately after (partial) metamorphosis (for review, see GREVEN, 1988). Teeth in adult paedomorphic *E. neotenes* therefore can be classified as late larval stage

Transformed adult plethodontids such as *Eurycea bislineata* (e.g., STEWART, 1958) or *Desmognathus fuscus* (NOBLE, 1931 and further references herein) show a remarkable difference in the premaxillary teeth of males and females. Depending on the testosterone level, males have more elongate and secondarily monocuspid teeth during the breeding period and a subsequent marked loss of these teeth. In addition, males lack the anterior vomerine dentition (NOBLE, 1931). Our few measurements and counts indicate that males of *E. neotenes* have longer teeth, mainly on the premaxillae. Further studies using more specimens captured in different seasons should confirm this observation and help clarify the regulation of this possible sex dimorphism.

Some other obviously sex-linked differences are worth noting. Males also possess broader premaxillae lacking a prominent *pars palatina*, fully separated *processus faciales*, larger anterior processes of the vomer, that overlap the parasphenoid by the fissured margins of the toothless palatal portion of the palatopterygoid, and large splenials.

According to DUTLI MAN & TRUEB (1985: 194), "*Euryceae neotenes* and *Typhlotriton spelaeus* are facultative neotenes intermediate ecologically between obligate neotenes and those species of *Eurycea* that undergo normal metamorphosis in surface waters" *E. neotenes* transforms not only following treatment with thyroxine, but also naturally (for literature see SWLET, 1977). Paedomorphosis, which includes neoteny, however, is evolutionarily fixed (GOULD, 1977). Therefore terms as "obligate paedomorphosis" and "facultative paedomorphosis" should be avoided (REILLY, 1994), but are in common use. As outlined by REILLY (1994), investigations regarding interspecific heterochrony ("patterns of phyletic change that have become fixed in independent phylogenetic lineages") and intraspecific heterochrony ("variation of individuals in response to environmental change") could help elucidate the paedomorphic state of *E. neotenes*. However, discussion on this is beyond the topic of our paper

The skull of metamorphosed individuals (see figure 6 in SWEET, 1997) corresponds in most respects to the skull of other transformed *Eurycea* as characterized by WAKE (1966) and MUTZ & CLEMEN (1992). As we studied maturing and mature *E. neotenes*, which reproduced in captivity (HAKIR, personal communication), we do not believe that the differences found between sexes should be attributed to different stages of development towards metamorphosis, but reflect real sex specific characters. We do not exclude, however, that an increasing number of breeding cycles (and, thus, briefly elevated thyroxine levels) may enhance metamorphic effects in older specimens as seen on other species (unpublished)

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